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Behavioral responses of tadpoles of *Duttaphrynus melanostictus* (Anura: Bufonidae) to cues of starved and fed dragonfly larvae

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Abstract

Behavioral responses of tadpoles of *Duttaphrynus melanostictus* (Anura: Bufonidae) to cues of starved and fed dragonfly larvae. Tadpoles of *Duttaphrynus melanostictus* use chemoreception to detect kairomonal cues and excretory metabolites from predatory anuran tadpoles (*Hoplobatrachus tigerinus*) that consume them. We describe here the behavioral responses of tadpoles of *D. melanostictus* to predatory dragonfly larvae (*Pantala flavescens*). The predator's kairomones (water conditioned by the starved predator) or its diet-derived metabolites released in excreta of predator after consumption of conspecific prey tadpoles were used to simulate predation risk. The tadpoles of *D. melanostictus* had no behavioral response to predator kairomones. However, the larvae reduced swimming movements and overall time spent in swimming, and had a higher burst speed/swimming velocity in response to water borne cues released from the excreta of predators fed conspecific prey. Thus, just the presence of dragonfly larvae does not elicit defense behaviors in tadpoles of *D. melanostictus*, but when predation risk is recognized as real (i.e., when tadpoles are exposed to excretory metabolites of predators fed conspecific tadpoles), defense behaviors are activated.

Keywords: Defense behavior, dietary cues, kairomones, *Pantala flavescens*, predator-prey interactions, tadpoles, Wandering Glider.

Resumo

Respostas comportamentais de girinos de *Duttaphrynus melanostictus* (Anura: Bufonidae) a sinais de larvas de libélula famintas e alimentadas. Os girinos de *Duttaphrynus melanostictus* usam a quimiorrecepção para detectar sinais cairomonais e metabólitos excretados por girinos predadores (*Hoplobatrachus tigerinus*) que os consomem. Descrevemos aqui as respostas comportamentais de girinos de *D. melanostictus* a larvas de libélulas predadoras (*Pantala flavescens*). Os cairomônios do predador (água condicionada pelo predador faminto) ou seus metabólitos derivados da dieta liberados nos excretas do predador após o consumo de girinos coespecíficos foram utilizados para simular o risco de predação. Os girinos de *D. melanostictus* não apresentaram resposta

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comportamental aos cairomônios dos predadores. Contudo, as larvas reduziram os movimentos de natação e o tempo total gasto na natação e apresentaram uma maior velocidade de partida e de natação em resposta a estímulos transmitidos pela água liberados dos excretas de predadores que se alimentaram de presas coespecíficas. Assim, apenas a presença de larvas de libélula não elicia comportamentos de defesa nos girinos de *D. melanostictus*, mas os comportamentos de defesa são ativados quando o risco de predação é reconhecido como real (isto é, quando os girinos são expostos a metabólitos excretados pelos predadores alimentados com girinos coespecíficos).

Palavras-chave: cairomônios, comportamento de defesa, estímulos alimentares, girinos, interações predador-presa, *Pantala flavescens*, planador-errante.

Introduction

In nature, predation is a significant selective force acting on prey that drives the evolution of strategies for assessment of predation risk and the development of antipredator defense strategies in order to optimize survival and fitness. Detection of predators before an encounter can give prey the opportunity to respond behaviorally and reduce the probability of being detected, and hence eaten (Lima and Dill 1990). However, inducing anticipatory antipredator behavior has direct energetic costs, as well as costs associated with reduced opportunity to feed, that affect growth and development of tadpoles (Lima and Dill 1990, Ferrari *et al.* 2010). Aquatic anuran tadpoles assess predation risk using chemosensory mechanisms before responding with defense behaviors (Saidapur *et al.* 2009, Ferrari *et al.* 2010, Mogali *et al.* 2012, 2015). Specifically, they perceive alarm cues released by injured prey, kairomones (chemicals originating from the body of predators), and dietary cues (excretory metabolites or substances of predators fed conspecific/heterospecific prey) to alter behavior to escape predation (Wisenden 2000, Hagman 2008, Schoepner and Relyea 2009a, Mogali *et al.* 2011, Carlson *et al.* 2015, Scherer and Smee 2016).

In southern India, most anurans including the common toad, *Duttaphrynus melanostictus* (Schneider, 1799), breed in ephemeral ponds following south-western monsoon rains. This leads to the larvae of several species coexisting sympatrically (Saidapur 2001). Some of these

tadpoles are carnivorous and even cannibalistic. For example, tadpoles of the Indian bullfrog, *Hoplobatrachus tigerinus* (Daudin, 1802), are voracious predators that hunt actively and also are cannibalistic (Saidapur 2001, Rajput *et al.* 2011). The ponds in this region also harbor many predatory aquatic insects such as water beetles, water scorpions, and dragonfly larvae. The larvae of the dragonfly *Pantala flavescens* (Odonata: Libellulidae) are “sit-and-wait” predators that move slowly and wait for the prey to approach before attacking. We have shown that toad tadpoles detect kairomones of tadpoles of *H. tigerinus*, as well as dietary metabolites or substances from a predator that consumed toad tadpoles (Saidapur *et al.* 2009, Mogali *et al.* 2011). Herein, we describe antipredator behavioral responses of tadpoles of *D. melanostictus* in the presence of dragonfly larvae.

Materials and Methods

Egg strings ($N = 4$) of *Duttaphrynus melanostictus* were collected from temporary ponds in and around the Karnatak University Campus, Dharwad, India (15.44° N, 74.98° E), in June 2010. In the laboratory, they were placed in separate plastic tubs (42 cm diameter and 16 cm depth) containing 10 L of aged tap water. All eggs hatched almost simultaneously at Gosner stage 19 (Gosner 1960) a day after their collection. Subsequently, hatchlings of the different clutches were mixed ($N = 100$ per clutch \times 4 clutches = 400 hatchlings) and reared in a glass aquarium (75 L \times 45 W \times 15 H cm)

with 15 L of aged tap water. From this stock, tadpoles of similar size (16.28 ± 0.80 mm total length) and developmental stage (Gosner stages 27–28) were randomly chosen for the experiments. The final instar larvae of *P. flavescens* ($N = 50$; 30.2 ± 1.3 mm total length) were obtained from the same ponds where the egg clutches were collected. Dragonfly larvae were individually reared in plastic bowls (14 cm diameter and 7 cm depth) with 200 mL of aged tap water, to avoid cannibalism. Tadpoles in the stock tanks were fed boiled spinach. Dragonfly larvae were fed toad tadpoles. The behavioral responses of the prey were studied by exposing them to “stimulus solution” of either predator kairomones or dietary metabolites of predators fed conspecific tadpoles. The preparation of stimulus solutions was, as follows.

Preparation of kairomones.—Individual dragonfly larvae were placed in separate plastic bowls ($N = 10$ bowls; 19 cm diameter and 7 cm depth) with 200 mL of aged tap water without food for 96 h to eliminate diet-derived excretory metabolites from the stimulus solution, resulting in a stimulus solution with only kairomones.

Preparation of dietary cues of conspecific origin.—Three dragonfly larvae were placed in plastic bowls ($N = 5$ bowls; 19 cm diameter and 7 cm depth) with 600 mL of aged tap water along with 12 toad tadpoles at Gosner stages 27–28 (at about 08:30 h). The dragonfly larvae consumed all the tadpoles provided to them by the evening (18:30 h). On the following day between 09:30 h and 11:30 h, predators were removed and the water from the bowls was filtered using fine cheese cloth. The filtrate served as the stimulus solution containing the diet-derived excretory metabolites or substances of predators fed conspecific prey and are unlikely to have contained the alarm cues of prey. Prey alarm cues are known to be labile in nature (Ferrari *et al.* 2008, Wisenden *et al.* 2009, Chivers *et al.* 2013). Thus, it is unlikely that prey alarm cues were present in the stimulus solution because all prey were consumed more than 15 h before the solution was collected.

Behavioral Responses of Prey to Predator's Kairomones

The behavioral responses of prey tadpoles to kairomones (water conditioned with starved predators) were recorded by placing single tadpole in a rectangular glass tank (28 L \times 15 W \times 15 H cm) with 600 mL of aged tap water. A handycam (Sony, DCR-SR300/E) was positioned above the tank to record activity in the entire tank. The handycam was connected to a computer with the Ethovision Video Tracking System (Noldus Information Technology, The Netherlands) to track movements of the tadpole before and after addition of stimulus solution to the test tank. The Ethovision system was used to record maximum swimming speed (V_{\max}), distance traversed by the tadpole, number of swimming spurts, and time spent swimming during an entire trial. For each trial, a tadpole put into the tank and left undisturbed for 5 min. A burette was placed ~ 1 cm above the water level and 50 mL of aged tap water (chemical blank) was then added at the rate of ~ 1 mL/s. The burette was then removed gently. Movement of the tadpole was then recorded for 5 min using Ethovision to record its baseline activity in the absence of any cues. After tracking baseline activity, 50 mL of stimulus solution containing kairomones was added as described above. Movement of the tadpole was recorded for another 5 min to determine the activity pattern after exposure to kairomones.

Behavioral Responses of Prey to Dietary Cues of Conspecific Origin

In this experiment, the stimulus solution contained chemical cues from excretory metabolites or substances of dragonfly larvae fed toad tadpoles instead of only kairomones of the predator. The behavioral responses of tadpoles were recorded as described above, before and after the addition of stimulus solutions.

In both experiments, a total of 25 trials were run using a new tadpole each time. The test tank was cleaned and replenished with aged tap water between trials.

Statistical Analysis

The data on V_{\max} , swimming spurts, time spent swimming and total distance traversed were log transformed prior to analysis to meet the assumption of normality. The behavioral responses of tadpoles before and after the addition of stimulus solutions (predator kairomones or diet-derived excretory metabolites) were compared using Paired-Samples t-test. Statistical tests were performed using SPSS ver. 16.0.

Results

The V_{\max} ($t = -0.373$, $df = 24$, $p = 0.712$, Figure 1A), frequency of swimming spurts ($t = 0.528$, $df = 24$, $p = 0.602$, Figure 1B), time spent swimming ($t = 0.126$, $df = 24$, $p = 0.901$, Figure 1C) and total distance traversed ($t = 0.123$, $df = 24$, $p = 0.903$, Figure 1D) by tadpoles exposed to chemical blank water were similar to those exposed to kairomones.

Upon exposure to diet-derived metabolites of a predator after it consumed conspecific prey, tadpoles showed a significant increase in V_{\max} ($t = -5.092$, $df = 24$, $p < 0.001$, Figure 2A), and significant declines in the number of swimming spurts ($t = 7.154$, $df = 24$, $p < 0.001$, Figure 2B), time spent swimming ($t = 7.242$, $df = 24$, $p < 0.001$, Figure 2C) and total distance moved ($t = 6.879$, $df = 24$, $p < 0.001$, Figure 2D) when compared to their baseline activities in stimulus-free water.

Discussion

Tadpoles of *Duttaphrynus melanostictus* do not alter their behavior in response to only kairomones of predatory larvae of *P. flavescens*, thereby suggesting that they do not perceive cues of the dragonfly larvae as a serious predation threat. Similar results have been reported for tadpoles of *Indosylvirana temporalis* (Günther, 1864) (Mogali et al. 2012) and *Euphlyctis cyanophlyctis* (Schneider, 1799) (Supekar and

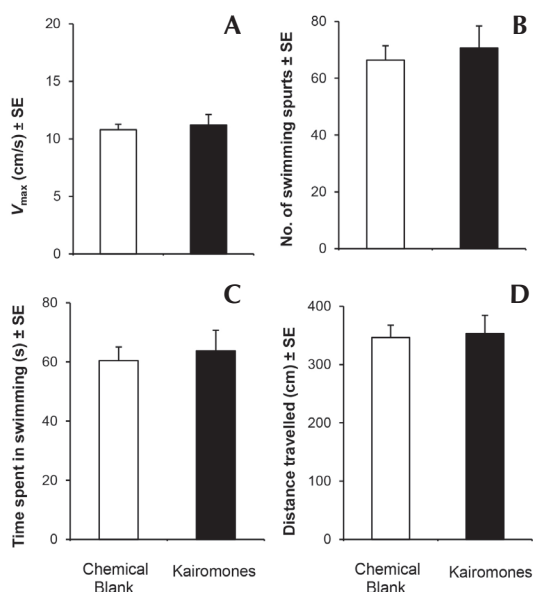


Figure 1. Maximum swimming speed (V_{\max}) (A), Swimming spurts (B), Time spent in swimming (C), and Distance travelled (D) by tadpoles of *Duttaphrynus melanostictus* exposed to chemical blank water (aged tap water) or kairomonal stimuli of *Pantala flavescens*. Data are represented as mean ± SE; $N = 25$ trials; data analyzed by Paired-Samples t-test.

Gramapurohit 2018). In contrast, toad tadpoles exhibit strong behavioral responses (i.e., reduced swimming movements and high burst speed) to kairomones of predatory sympatric tadpoles of *Hoplobatrachus tigerinus* (Mogali et al. 2011), which locate prey visually and prey on coexisting sympatric anuran tadpoles (Saidapur 2001, Saidapur et al. 2009). Thus, frog species such as *H. tigerinus* pose a serious predation threat. The long ecological coexistence of toad tadpoles with sympatric carnivorous tadpoles such as *H. tigerinus* may have led to the evolution of antipredator defense strategies in response to kairomones of these predators.

In contrast, larvae of dragonfly are “sit-and-wait” predators that move slowly and usually wait for prey to come near before attacking

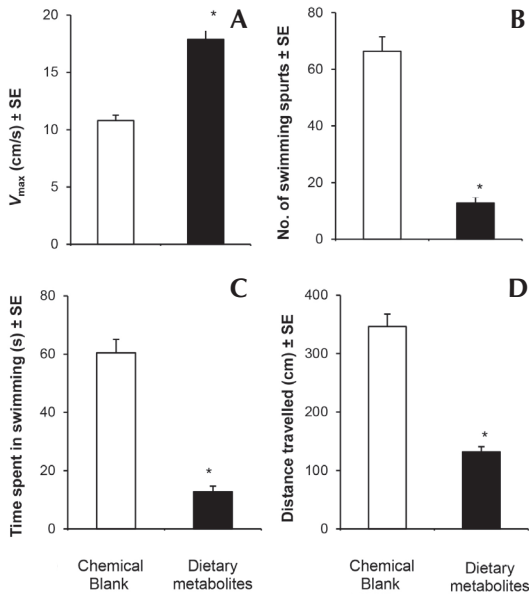


Figure 2. Maximum swimming speed (V_{max}) (A), Swimming spurts (B), Time spent in swimming (C), and Distance travelled (D) by tadpoles of *Duttaphrynus melanostictus* exposed to chemical blank water (aged tap water) or dissolved diet-derived excretory metabolites of conspecific prey fed *Pantala flavescens*. Data are represented as mean \pm SE; $N = 25$ trials; data analyzed by Paired-Samples t-test. Asterisks over the bars indicate significant difference between chemical blank water and diet-derived metabolites of predators from excreta groups.

(Miller *et al.* 2014). They seem to be perceived by the prey as less dangerous and they may pose a lower predation threat. There is intense selection pressure on “sit-and-wait” predators to suppress chemical (e.g., kairomones) evidence of their presence (Miller *et al.* 2015) because they need the prey to approach them closely. Also, it is possible that the predation pressure of dragonfly larvae on toad tadpoles is low. If so, toad tadpoles are better served by conserving their energy by not inducing antipredator defenses in response to kairomones, if any, of dragonfly larvae. In contrast, water-soluble

substances in the excreta of larvae of *P. flavescens* following consumption of conspecific prey seems to indicate intense predation threat and elicit strong behavioral changes in toad tadpoles in the form of reduced swimming movements and high burst speed. Apparently, evidence of recent predation on conspecific members of the group indicates high predation risk for tadpoles in the vicinity.

Our results are consistent with those reported for tadpoles of *Dryophytes versicolor* (LeConte, 1825) (Schoeppner and Relyea 2009b). We rule out the influence of alarm cues released by toad tadpoles as there were neither surviving injured individuals nor dead remains of tadpoles in the bowls for 15 h prior to harvesting the stimulus solution, and alarm cues are highly labile in nature (Ferrari *et al.* 2008, Wisenden *et al.* 2009, Chivers *et al.* 2013). Hence, the antipredator defense behavior of toad tadpoles in the present study is specifically in response to diet-derived excretory metabolites or substances released by dragonfly larvae that have consumed conspecific prey. In conclusion, toad tadpoles can exhibit different behavioral responses to different predator-related cues.

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